

WHAT IS CLAIMED IS:

1. A method of fluorescence-based cycle sequencing of a sample DNA, comprising,

(a) preparing a reaction mixture containing:

- (i) the sample DNA,
- (ii) a primer set complementary to DNA primer sites flanking or interspersed within the sample DNA, wherein the T<sub>d</sub> of the primers in the primer set are between about 72 °C and 75 °C,
- (iii) a thermostable polymerase,
- (iv) a mixture of dNTPs and fluorescently-labeled ddNTPs, and
- (v) a suitable buffer

(b), dissociating the sample DNA to create single stranded templates, wherein said dissociation is achieved by heating the DNA to between about 92 °C and 95 °C for at least about 3 minutes;

(c) annealing the primers to the primer sites, wherein said annealing is achieved at a temperature of between about 65°C and 67°C for at least about 30 seconds;

(d) extending the annealed primers to generate a series of fluorescently-labeled dideoxynucleic acid fragments, wherein said primer extension is achieved at a temperature of between about 75°C and 78°C for between about 3 to 4 minutes;

(e) heating the reaction mixture to between about 92°C and 95°C in order to dissociate double stranded DNA;

(f) repeating the steps c through e for a plurality of cycles; and

(e) determining the nucleotide sequence of the sample DNA from the series of fluorescently-labeled dideoxynucleic acid fragments present in the reaction mixture.

2. The method according to claim 1, wherein the number of cycles is between about 30 and 50 cycles.

3. The method according to claim 1, wherein the number of cycles is between about 50 and 60 cycles.

4. The method according to claim 1, wherein the number of cycles is between about 60 and 70 cycles.

5. The method according to claim 1, wherein the the primers are complementary to a PUC18 vector containing the sample DNA and have the following nucleotide sequences:

5' GCT GCA AGG CGA TTA AGT TGG GTA 3' (SEQ ID NO: 1)

5' GTT GTG TGG AAT TGT GAG CGG ATA AC 3' (SEQ ID NO: 2)

6. The method according to claim 5, wherein primer annealing is achieved at 67°C for 30 seconds, and primer extension is achieved at 75°C for 4 minutes.

7. The method according to claim 1, wherein the thermostable DNA polymerase is a *Taq* polymerase.

8. The method according to claim 1, wherein the *Taq* polymerase contains a F667Y point mutation.

9. A method of sequencing a GC-rich DNA sample on an automated fluorescence-based cycle sequencer, comprising

(a) providing primers having a Td of between about 73°C and 74°C in a dye-terminator sequencing reaction comprising the DNA sample, a *Taq* polymerase and dNTPs and fluorescently-labeled ddNTPs, in a suitable buffer, under substantially the following cycle conditions:

*Step 1 = 3 min @ 92 °C*  
*X 1 cycle*

*Step 2 = 30 sec @ 92 °C*  
*30 sec @ 67 °C*  
*4 min @ 75 °C*  
*X 60 cycles*

*Step 3 = soak @ 4 °C*

(b) determining the nucleotide sequence of the DNA sample.

10. A method of sequencing a DNA sample containing CCT repeats on an automated fluorescence-based cycle sequencer, comprising

(a) providing primers having a Td of between about 57°C and 75°C in a dye-terminator sequencing reaction comprising the DNA sample, a *Taq* polymerase and dNTPs and fluorescently-labeled ddNTPs, in a suitable buffer, under substantially the following cycle conditions:

*Step 1 = 1 min @ 92 °C*  
*X 1 cycle*

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*Step 2 =      15 sec @ 92 °C*  
*10 sec @ 54 °C*  
*4 min @ 65 °C*  
*X 60 cycles*

*Step 3 =      soak @ 4 °C*

(b) determining the nucleotide sequence of the DNA sample.